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Analysis of Stomata Distribution Patterns for Quantification of the Foliar Plasticity of Tradescantia Zebrina

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Abstract. Here we propose a method for the analysis of the stomata distribution patterns on the surface of plant leaves. We also investigate how light exposure during growth can affect stomata distribution and the plasticity of leaves. Understanding foliar plasticity (the ability of leaves to modify their structural organization to adapt to changing environmental resources) is a fundamental problem in Agricultural and Environmental Sciences. Most published work on quantification of stomata has concentrated on descriptions of their density per unit of leaf area, however density alone does not provide a complete description of the problem and leaves several unanswered questions (e.g. whether the stomata patterns change across various areas of the leaf, or how the patterns change under varying observational scales). We used two approaches here, to know, multiscale fractal dimension and complex networks, as a means to provide a description of the complexity of these distributions. In the experiments, we used 18 samples from the plant Tradescantia Zebrina grown under three different conditions (4 hours of artificial light each day, 24 hours of artificial light each day, and sunlight) for a total of 69 days. The network descriptors were capable of correctly discriminating the different conditions in 88% of cases, while the fractal descriptors discriminated 83% of the samples. This is a significant improvement over the correct classification rates achieved when using only stomata density (56% of the samples).

1. Introduction

Phenotypic plasticity is the ability of an organism to change its behaviour or structural organization to cope with the surrounding environment [1]. This is an important characteristic of plants, considering their immobility, in that it facilitates the survival in inhospitable environments such as those with inadequate temperatures or amount of light and nutrients.

Understanding where and how plasticity works in plants is an important challenge in the context of the climate changes observed in recent times. Plasticity can modulate organism structure faster than natural evolution and hence play a fundamental role in the adaptation process. Furthermore changes in plant behaviour may reflect the nature of environmental changes (e.e. pollution levels), or pathologies, where an understanding of plasticity might provide clues for disease diagnosis and prognosis.

Leaves are important interfaces between the plant and the environment and are very susceptible to plasticity. This may be expressed by external changes such as size, shape, thickness or by changes in the organization of their structure. Among the latter, the relation between the

stomata density and the plasticity is well known. For instance, plants from the same species grown under different amounts of light tend to vary in the density of stomata on the leaf surface. However, their distribution is not fully described only by the density. In this context, we propose a mathematical-physical approach to describe such distribution in a more complete and precise way. The proposed analysis was performed over samples from species Tradescantia Zebrina, as the stomata can be easily identified in leaves from that species.

2. Method

Two state-of-the-art approaches in shape analysis were investigated: multiscale fractal dimension and complex networks.

2.1. Multiscale fractal dimension

Fractal descriptors provide multiple scale features of an image or point set considered to be an approximation of a fractal set. This modelling is suitable to analyse complex biological morphologies, including the stomata distribution considered here, that exhibit high degrees of complexity.

We propose using a method called 'multiscale fractal dimension' (MFD), originally applied to shape analysis in [2], but generalized to sets of disjoint points in the current application. Essentially, this consists of simultaneously dilating all points in the set with circular structural elements of radius r and then computing the total area of the dilated structure A(r). Finally, the descriptors are computed from the derivative of the function $\log(r) \times \log(A(r))$ when r is ranged within an interval of values. Here r ranges from 1 to 100 pixels.

2.2. Complex networks

Complex networks is an area of study combining Graph Theory with analytical tools from Statistical Physics [3]. The essential idea is to model a problem structure in terms of a graph to then extract measures from that graph, which then can be used as features to represent the modelled system.

In the case when a set of points is to be analysed, the strategy adopted in [4] can be adapted by considering each set point as a graph vertex and the Euclidean distance between points as a graph edge weight. Likewise in [4] we follow an approach named dynamic diffusion, where starting from a complete graph (with all vertices are connected) a threshold function $f(G;t)$ is applied to remove all edges with weights smaller than t.

When a range of values of t is used, one network is generated for each threshold and measures can be computed for each of those networks and used as features characterising the original set of points. Here a range of Euclidean distances between 1 and 100 was applied and the average degree (number of vertices connected to each node) was computed for each thresholded network.

3. Results

Fractal and complex network descriptors were extracted from the distribution of stomata over the leaf surface from the plant Tradescantia Zebrina under different illumination conditions: sunlight, 4 hours of artificial light every day and 24 hours of artificial light every day, during 69 days in a greenhouse with controlled conditions. The images were collected by using a Zeiss discovery V.20 stereo microscope coupled to an Axiocam ICc1 camera. Figure 1 shows images of three leaves (one for each light condition) and the stomata manually identified as points in a binary image while figure 2 shows the average descriptors (complex networks and fractal) for each light condition. To discriminate between the conditions, both descriptor types were submitted to a 2-Nearest-Neighbour classifier using χ^2 distance and following a leave-one-out scheme [5].

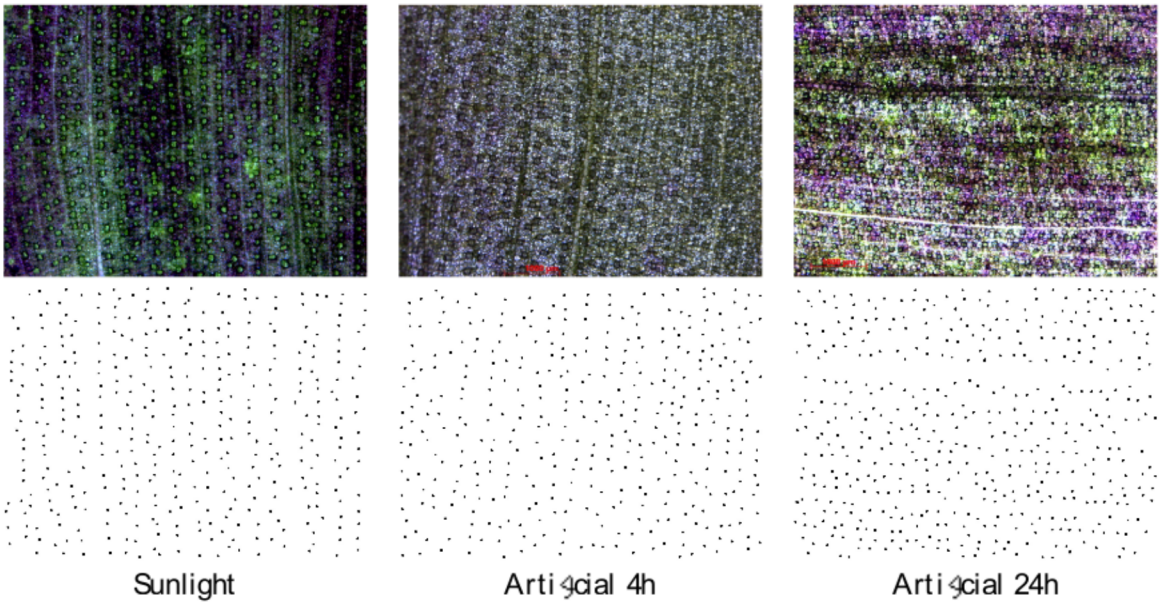

Figure 1. Examples of Tradescantia Zebrina leaf samples and respective stomata distribution.

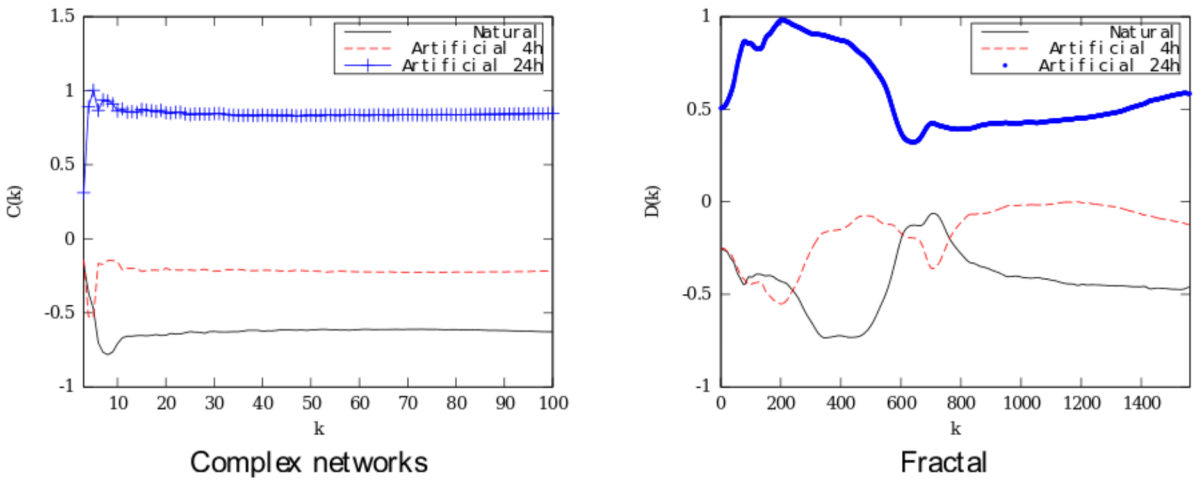

Figure 2. Average Descriptors (z-score normalized) of stomata distribution in each light condition.

Figure 3 shows the rates of correct classification when using varying numbers of descriptors in both fractal and network modelling. Twenty-one fractal descriptors were sufficient to correctly discriminate 83.3% of the samples (15 samples), whereas when 62 network descriptors were used, the discrimination increased to 88.9% (16 samples). This result significantly improves the classification results obtained when considering only stomata density (55.6% correct). Table 1 shows the respective confusion matrices. We observe that it is possible to separate samples of plants grown under sunlight from those under artificial light. However, when only the exposition hours to artificial light is taken into account, the difference in stomata organization appears to be more subtle. Both approaches reveal that stomata patterns on leaves can be successfully quantified and that detectable differences appear under the various experimental conditions investigated here. These preliminary results suggest that it might be possible to reveal the

effects of other environmental factors such as pollution or diseases affecting forests or crops, hence providing more quantitative tools to allow precise planning in the detection and solution of such problems.

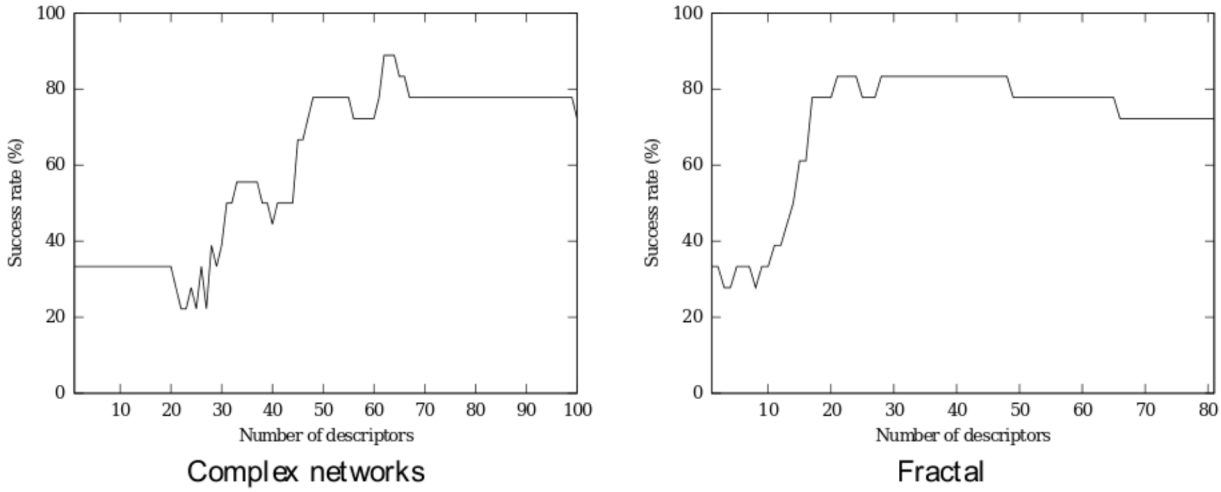

Figure 3. Correctness rates.

		Predicted		
		S	A4h	A24h
Actual	S	6	0	0
	A4h	3	2	1
	A24h	1	3	2

Density

		Predicted		
		S	A4h	A24h
Actual	S	6	0	0
	A4h	1	5	0
	A24h	1	0	5

Complex networks

		Predicted		
		S	A4h	A24h
Actual	S	6	0	0
	A4h	2	4	0
	A24h	1	0	5

Fractal

Table 1. Confusion matrices. S = Sunlight. A4h = Artificial light for 4 hours. A24h = Artificial light for 24 hours.

4. Conclusions

We showed a systematic representation and quantification of the stomata distribution over the leaf surfaces of (Tradescantia Zebrina) by means of two state-of-the-art shape analysis methods, namely the multiscale fractal descriptors and complex networks. The results showed that both approaches were capable of discriminating between three different experimental lighting conditions used during the plant growth.

The results confirm that the amount and type of exposition light influences the stomata arrangements in the leaves. Furthermore, although the stomata density could identify the samples grown under sunlight, our analysis was able to discriminate between samples grown under 4 and 24 hours of artificial light a day. These results exemplify how imaging methods can help in quantify and elucidating the relationships between plants and the environment.

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